iGEM2014 – Microbiology – BMB – SDU	
Title: Ligation **SOP number:** SOP0015_v01 **Version number:** 01	**Date issued:** 2013.06.19 **Review date:** 2015.09.13 **Written by:** ASF

1. Purpose

To ligate pieces of DNA

2. Area of application

Cloning

3. Apparatus and equipment

Apparatus/equipment	Location (Room number)	Check points	Criteria for approval/rejection
Vortex	Laboratory 1. Floor	●	
Pipettes (p20, p10)	Micro Storage	●	
		●	
		●	
		●	
		●	
		●	

4. Materials and reagents – their shelf life and risk labelling

Name	Components	Supplier / Cat. #	Room (hallway storage)	Safety considerations
Purple pipette tips		Contact lab-manager	Micro storage	
Green pipette tips		Contact lab-manager	Micro storage	
Eppendorftubes		Contact lab-manager	Micro storage	
Distilled water		Contact lab-manager	Micro storage	
Ligasebuffer		Agilent Technologies	Freezer at 1. Floor	
Ligase			Freezer 1. Floor	

DNA piece 1			Refrigiator 1. Floor	
DNA piece 2			Refrigiator 1. Floor	

5. QC – Quality Control

6. List of other SOPs relevant to this SOP

7. Environmental conditions required

8. Procedure

1. Prepare the ligation mixture and mix by pipetting up and down
2. Leave the mixture overnight at 16°C

2a. If there is no time leave the ligation solution at 22.5°C for 30mins. Then denature the ligase at 65°C for 10min.

3. Use ligation solution for transformations

Reagents	**Volume**
10x T4 DNA ligase buffer	2 µL
T4 DNA ligase (add last!)	1 µL
PCR product (cut) of each brick which is to be ligated – **or** 1 part plasmid and 5 part bricks	5 µL **or** 10 fmol Plasmid, 0, 10 and 20 fmol PCR
H2O	to reach a total volume of 20µL

9. Waste handling

Chemical name	Concentration	Type of waste (C, Z...)	Remarks
One use Plastic		GMO	Yellow GMO Trash

10. Time consumption

- 3 Hours
- 1 Hour + Ligation overnight

11. Scheme of development

Date / Initials	Version No.	Description of changes
13.06.19 / ASF	01	The SOP has been written
13.06.26 /PRA	01	The SOP has been approved

12. Appendixes